Long-term caffeine consumption exacerbates renal failure in obese, diabetic, ZSF1 (fa-fa^{cp}) rats

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Long-term caffeine consumption exacerbates renal failure in obese, diabetic, ZSF1 (fa-fa^{cp}) rats.

Background. Our preliminary studies indicate that chronic caffeine consumption has adverse renal effects in nephropathy associated with high blood pressure and insulin resistance. The purpose of this study was to investigate the effects of early (beginning at 8 weeks of age) and long-term (30 weeks) caffeine treatment (0.1% solution) on renal function and structure in obese, diabetic ZSF1 rats.

Methods. Metabolic and renal function measurements were performed at six-week intervals and in a subset of animals ($N = 6$ per group) heart rate (HR) and mean arterial blood pressure (MABP) were monitored by a radiotelemetric technique. At the end of the study acute, measurements of renal hemodynamics and excretory function were conducted in anesthetized animals.

Results. Caffeine produced a very mild increase (4 to 5%) of MABP and HR, but greatly augmented proteinuria ($P < 0.001$), reduced creatinine clearance ($P < 0.05$) and had a mixed effect on metabolic status in obese ZSF1 rats. Caffeine significantly reduced body weight, glycosuria, fasting glucose and insulin levels and improved glucose tolerance, had no effect on elevated plasma triglycerides levels and significantly increased plasma cholesterol level ($P < 0.001$). Acute measurements of renal function revealed increased renal vascular resistance (95.1 ± 11 vs. 50.7 ± 2.4 mm Hg/mL/min/g kidney, $P < 0.01$) and decreased inulin clearance (0.37 ± 0.11 vs. 0.97 ± 0.13 mL/min/g kidney, $P < 0.002$) in caffeine-treated versus control animals, respectively. Caffeine potentiated the development of more severe tubulointerstitial changes ($P < 0.05$) and increased focal glomerulosclerosis (14.7 ± 1.7 vs. $6.5 \pm 0.9\%$, caffeine vs. control, $P < 0.002$).

Conclusion. The present study provides the first evidence that caffeine (despite improving insulin sensitivity) exacerbates renal failure in obese, diabetic ZSF1 rats. Further mechanistic studies of adverse renal effects of caffeine in chronic renal failure associated with metabolic syndrome are warranted.

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Caffeine is the most widely used drug in the world, and any reported benefits or risks of caffeine consumption have received considerable attention [1, 2]. Any adverse health consequences associated with caffeine ingestion are of importance in view of the compound's widespread use. However, the effects of caffeine consumption on renal function have received little attention. This is surprising, particularly in view of the fact that caffeine is present in most analgesic combination drugs in which long-term use may lead to analgesic nephropathy.

In Western societies, obesity is frequently associated with the metabolic syndrome (that is, insulin resistance, hyperlipidemia and hypertension), and this triad carries a high risk for renal disease with at least 70% of end-stage renal failure being attributed to hypertension or diabetes [3–6]. Nevertheless, no information is available regarding the metabolic and renal effects of chronic caffeine consumption in the setting of obesity and the metabolic syndrome.

Although there have been no clinical or epidemiological studies evaluating the impact of caffeine in chronic renal failure, limited experimental [7–9] and clinical data [10, 11] suggest that adenosine may have beneficial effects in both experimental and human chronic renal failure, including diabetic nephropathy. Caffeine is a nonselective adenosine receptor antagonist and at physiological concentrations (that is, 2 to 10 μg/mL, plasma levels seen in humans after moderate to heavy intake of coffee) most, if not all, of caffeine's effects are mediated via blockade of adenosine A_1 and A_{2A} receptors [12–14]. Since caffeine is an adenosine receptor antagonist, it might be expected that caffeine would oppose the beneficial effects of adenosine in the nephropathic kidney. This reasoning prompted us to perform studies of caffeine consumption in several experimental models of nephropathy [15–17].

Our preliminary studies in lean animals indicated that chronic caffeine consumption accelerates the development of nephropathy in the setting of high blood pressure and insulin resistance [16]. Furthermore, very recently

we have noticed that caffeine consumption for 8 weeks was associated with an accelerated decline in renal function in ZSF1 rats [17], an animal model that in addition to being obese is diabetic, hyperlipidemic, and hypertensive, and develops nephropathy [18]. However, caffeine did not potentiate renal histopathological changes. The absence of histopathological changes may be due either to the fact that adult, obese ZSF1 rats at baseline have significant renal histopathology [17], or that the treatment duration for caffeine must be earlier and/or longer to produce significant renal structural changes. Accordingly, the purpose of this study was to investigate the effects of early, long-term caffeine consumption on metabolic status and renal function and structure, in obese ZSF1 rats.

In the present study, physiologically relevant doses of caffeine that produce plasma caffeine concentrations of 5 to 10 μg/mL (similar to those seen in humans after modest to heavy caffeine consumption [7–9]) were utilized to test the hypothesis that chronic caffeine consumption adversely affects renal function in an experimental model of nephropathy associated with obesity, hypertension and the metabolic syndrome.

METHODS

Animals

A total of 24 young (8 weeks of age, body weight 285 ± 5 g), obese, male ZSF1 rats (Genetic Models Inc., Indianapolis, IN, USA) were used in this study. The obese ZSF1 rat model was developed by crossing lean female Zucker Diabetic Fatty rats (ZDF [19, 20]) with lean male Spontaneously Hypertensive Heart Failure rats (SHHF/Mcc-fa^cp [21]). We recently evaluated renal function in this model. In addition to being obese, these animals are hypertensive, hyperlipidemic, diabetic and develop marked renal dysfunction [18]. Also, we determined that obese ZSF1 rats do not develop some of the complications common for the parental strains (that is, hydronephrosis in ZDF and overt congestive heart failure in SHHF rats [20, 21]) that may compromise studies of renal function and structure. Rats were housed in the University of Pittsburgh Medical Center animal care facility (temperature, 22°C; light cycle, 12 hours; relative humidity 55%). Animals were fed Pro Lab RMH 3000 rodent diet (PMI Nutrition Inc., St. Louis, MO, USA) and were given water ad libitum. Institutional guidelines for animal welfare were followed, and experimental protocols were approved by the Institutional Animal Care and Use Committee.

Metabolism cage studies

Before, and 12, 18 and 24 weeks into the treatments, rats were placed in metabolic cages, and after a 24-hour acclimatization period, 24-hour urine volume and food

and water intake were measured. Rats were removed from the metabolic cages and weighed, and blood samples were drawn from tail vein and used for determination of plasma sodium potassium and creatinine concentrations. Plasma and urine samples were analyzed for sodium and potassium concentrations using a flame photometer (Model IL-943; Instrumentations Laboratory Inc., Lexington, MA, USA) and a creatinine analyzer (Creatinine Analyzer 2; Beckman Instruments, Inc., Fullerton, CA, USA) was used to measure plasma and urine creatinine. Total protein concentration was measured in the urine samples by a spectrophotometric assay using bicinchoninic acid reagent (Pierce, Rockford, IL, USA) and a modification of the Lowry et al's method [22]. Urine albumin concentration was measured by an enzyme-linked immunosorbent assay (ELISA) highly specific for rat albumin (Nephrat; Exocell Inc., Philadelphia, PA, USA), and urine glucose concentrations were measured by a spectrophotometric assay using the Infinity™ Glucose Reagent (Sigma Diagnostics, St. Louis, MO, USA).

After the baseline metabolism studies (week 0) were completed, animals were randomly assigned to drink tap water or a 0.1% caffeine solution. The selected dose of caffeine (0.1% in drinking water) previously has been shown to significantly attenuate depressor responses to adenosine and to provide plasma caffeine concentrations of approximately 10 μg/mL [23], which was equivalent to the plasma concentrations seen in humans after a modest intake of coffee (1 to 3 cups).

Metabolic status measurements

Blood samples for measurement of glucose, insulin and lipids were taken after 8, 17 and 26 weeks of treatment. After overnight fasting, between 8:00 and 10:00 AM, animals were anesthetized with halothane. One milliliter of blood was drawn from the tail vein, and plasma was frozen at −70°C.

Plasma samples were analyzed in duplicates for triglycerides and cholesterol levels (Sigma Diagnostics). Insulin levels were measured in duplicate by a double antibody radioimmunoassay specific for rat insulin (Incstar Corp., Stillwater, MN, USA). A drop of blood was used to measure blood glucose levels with the Precision Q.I.D. Blood Glucose Test Strips kit (Medisense, Inc., Bedford, MA, USA). After 26 weeks of treatment and overnight fasting, an oral glucose tolerance test was conducted. Blood samples for measurements of fasted glucose were taken, and animals were given glucose (2 g/kg/4 mL) by oral gavage. Blood withdraws were repeated after 30, 60 and 120 minutes.

Blood pressure measurements in conscious rats

Ten weeks into the treatment, radiotelemetry devices (Model TA11PA-C40; Data Sciences International, St. Paul, MN) were attached to a subset of animals (N = 6

per group) to permit monitoring of blood pressure and heart rate in conscious rats. The radiotelemetry device was inserted with the animal under halothane anesthesia as described previously [24, 25]. Animals received a postoperative IM injection of penicillin G procaine (5000 units) and heparin (50 units.) Data acquisition was performed using the Dataquest Lab Pro software package (Data Science International) with sampling parameters adjusted to 10-second scan periods at 10-minute intervals. Blood pressure and heart rate readings during the first week after the surgery were not included in the final analysis of data.

Acute measurements of renal function

At 38 weeks of age, after the 30-week treatment period, each rat was anesthetized with pentobarbital (45 mg/kg IP), and a short section of PE-240 polyethylene catheter was inserted into the trachea to facilitate breathing. The left carotid artery was cannulated with a PE-50 catheter for blood sample collection and mean arterial blood pressure (MABP) measurement via a digital pressure analyzer (Micro-Med. Inc., Louisville, KY, USA). Two PE-50 cannulas were placed in the left jugular vein: line A for infusion of ^{14}C-inulin (0.035 μCi/20 μL saline/min) and line B for infusion of saline (50 μL/min) or caffeine. Intravenous infusion of caffeine (2 mg/h, 20 μL/min) was initiated in animals that were chronically receiving caffeine to maintain plasma caffeine concentrations during the time course of the acute experiment. In a previous study (data not published), we observed that, in animals chronically treated with caffeine (0.1% in drinking water) the abstinence from caffeine for five hours during an acute experiment results in a decrease of caffeine plasma concentrations from ~10.9 μg/mL to ~0.17 μg/mL. This observation is congruent with the reported half-life of caffeine in rats ($t_{1/2}$ = 0.8 hours) [26]. Furthermore, in animals that were chronically consuming caffeine (0.1%), intravenous infusion of caffeine (2 mg/h) during the acute experiment provided plasma caffeine concentrations that blocked the effects of exogenously administered adenosine [27]. Next, a midline abdominal incision was made and the left kidney was exposed. A PE-10 catheter was inserted into the left ureter to facilitate collection of urine, and a flow probe (Model 1RB; Transonic Systems, Inc., Ithaca, NY, USA) was placed on the left renal artery for determination of renal blood flow (RBF).

A one-hour stabilization period was permitted before two 30-minute clearance periods [that is, baseline and after angiotensin-converting enzyme (ACE) inhibition] were conducted. MABP and RBF were recorded at 5-minute intervals, and averaged during a 30-minute urine collection. A mid-point blood sample (300 μL) to measure radioactivity, sodium and potassium was collected. Urine volume (UV) was determined gravimetri-

cally, and urine samples were analyzed for sodium and potassium. Plasma and urine ^{14}C-inulin radioactivity was measured (Liquid scintillation analyzer, Model 2500TR; Packard Instrument Company, Downers Grove, IL, USA), and renal clearance of ^{14}C-inulin was calculated as an estimate of glomerular filtration rate. Excretion rates of sodium and potassium and renal vascular resistance (RVR) were calculated. Next, a bolus dose of captopril (100 mg/kg/2 min) was administered, and 45 minutes later another 30-minute clearance period was conducted. Changes in renal hemodynamic and excretory function parameters after ACE inhibition were calculated in absolute values and as percent change from the baseline period. Animals were euthanized by anesthetic overdose. Right kidneys were removed and processed for histopathological analysis.

Renal histopathology

The right kidney tissue sample stored in 10% formalin buffer was sectioned and then processed into paraffin blocks for light microscopy. Two histological sections (3 μ thick) were cut and stained with hematoxylin-eosin (H&E) and methenamine silver-trichrome (MST). Kidney slices were examined by light microscopy and were scored in a blinded fashion by one of the investigators (S.B.). A total of at least 150 glomeruli from each rat were studied and the percentage of glomeruli showing segmental (FSGS) and global (FGGS) glomerulosclerosis was determined. Other histopathological features assessed semiquantitatively included tubular atrophy (0 to 3$^+$), interstitial inflammation (0 to 3$^+$), interstitial fibrosis (0 to 3$^+$), tubular dilation (0 to 4$^+$), arterial medial hypertrophy (0 to 3$^+$), and arteriolar sclerosis (0 to 3$^+$).

Statistical analysis

All data are presented as mean $\pm$ SEM. Statistical analyses were performed using the Number Cruncher Statistical software program (Kaysville, UT, USA). Group comparisons for data from metabolic studies (repeated measurements) were performed using a one (1F) or two (2F) hierarchical analysis of variance (ANOVA) as appropriate, followed by a Sher's LSD test for post-hoc comparisons. Comparison of data from acute experiments (single point data) was performed by the Student *t* test, and data from histopathological analysis were compared by non-parametric Mann-Whitney *t* test. The probability value of $P < 0.05$ was considered statistically significant.

RESULTS

The caffeine and control groups did not differ with regard to baseline (week 0) measurements of body weight, food and water intake, urine output and urinary protein excretion or creatinine clearance (Table 1).

Table 1. Metabolic effects of chronic caffeine consumption in obese (fa/fa^{cp}), diabetic ZSF1 rats

Parameter	0 week	6 weeks	12 weeks	18 weeks	24 weeks	2F-ANOVA P
Body weight *g*						
Control	284 ± 8	517 ± 8	588 ± 7	657 ± 10	704 ± 15	<0.001
Caffeine	290 ± 9	469 ± 6[a]	517 ± 6[a]	580 ± 9[a]	621 ± 14[a]	
Food intake *g/kg/day*						
Control	126 ± 13	82 ± 4	61 ± 1	55 ± 3	61 ± 1	<0.001
Caffeine	109 ± 9	61 ± 3[a]	50 ± 2[a]	47 ± 2[a]	49 ± 3[a]	
Fluid intake *mL/kg/day*						
Control	181 ± 14	205 ± 15	117 ± 7	133 ± 15	111 ± 8	<0.001
Caffeine	196 ± 15	124 ± 15[a]	80 ± 6[a]	89 ± 6[a]	86 ± 5[a]	
Urine volume *mL/kg/day*						
Control	103 ± 13	194 ± 10	91 ± 8	95 ± 9	86 ± 5	<0.001
Caffeine	106 ± 14[a]	117 ± 11[a]	61 ± 7[a]	76 ± 5[a]	63 ± 5[a]	
Urine glucose *g/day*						
Control	ND	7.3 ± 0.4	4.0 ± 0.5	3.6 ± 0.6	2.1 ± 0.5	<0.003
Caffeine	ND	5.2 ± 0.7[a]	2.3 ± 0.4[a]	2.7 ± 0.5[a]	1.6 ± 0.4[a]	

Parameter	8 weeks	17 weeks	26 weeks	2F-ANOVA P
Plasma glucose *mg/dL*				
Control	179 ± 15	171 ± 7	205 ± 11	<0.001
Caffeine	119 ± 15[a]	129 ± 3[a]	156 ± 8[a]	
Plasma insulin *μU/mL*				
Control	101.7 ± 3.4	97.7 ± 2.9	88.2 ± 6.0	<0.001
Caffeine	65.0 ± 6.1[a]	71.7 ± 3.2[a]	69.9 ± 9.4[a]	
Plasma triglycerides *mg/mL*				
Control	586 ± 52	705 ± 48	815 ± 40	NS
Caffeine	588 ± 27	779 ± 39	765 ± 58	
Plasma cholesterol *mg/dL*				
Control	207 ± 7	189 ± 13	225 ± 8	<0.005
Caffeine	259 ± 13[a]	282 ± 34[a]	628 ± 102[a]	

[a] $P < 0.05$ vs. Control, Fisher's LSD test

The effects of caffeine consumption on heart rate and blood pressure in conscious rats are presented in Figure 1. Caffeine consumption for 30 weeks significantly increased the heart rate and near the end of the study (weeks 20 to 29) produced a mild, but persistent and significant increase in blood pressure (4 to 6 mm Hg; $P < 0.05$; Fig. 1).

The metabolic effects of chronic caffeine consumption are presented in Table 1. Caffeine consumption reduced food intake (treatment effect $P < 0.001$) and attenuated the time-dependent increase in body weight ($P < 0.001$). At the end of the study, the control group and caffeine group weighted 740 ± 11 and 650 ± 20 g, respectively. The reduced weight gain was accompanied by significantly lower fasting glucose and insulin levels ($P < 0.001$), significantly reduced glycosuria ($P < 0.003$), and an improved glucose tolerance test conducted after 26 weeks of treatment (Fig. 2). The improved glucose homeostasis also was accompanied by reduced polydipsia and polyuria (Table 1). Caffeine had no effect on triglycerides levels, yet significantly increased total cholesterol levels ($P < 0.005$). A striking effect of caffeine consumption on urinary protein excretion was detected. In 14-week-old control animals, as obesity and the metabolic syndrome fully developed, there was a fivefold increase

in urinary protein excretion (UPE) as compared with baseline values, and proteinuria continued to increase with aging (Fig. 3A). Caffeine consumption for only six weeks doubled the UPE as compared with control animals, and this effect persisted until the end of the study. Measurement of urine albumin also revealed doubled albumin excretion in caffeine treated animals as early as six weeks into the treatment (Fig. 4). Creatinine excretion was lower in animals that were consuming caffeine (treatment effect, $P < 0.001$; Fig. 5A), and after 18 and 24 weeks of treatment the caffeine group had a significantly reduced creatinine clearance as compared with control animals (Fig. 5B).

In animals consuming caffeine for 30 weeks, acute measurements of renal hemodynamics and excretory function revealed reduce renal blood flow (RBF), UV, and glomerular filtration rate (GFR; that is, inulin and creatinine clearance) and increased renal vascular resistance (RVR), plasma creatinine levels and urinary protein excretion (Table 2). Captopril produced a greater reduction in RVR and greater increase in RBF and UV in caffeine-treated as compared with control animals (Fig. 6), suggesting an increased activity of the renin-angiotensin system in animals that consumed caffeine.

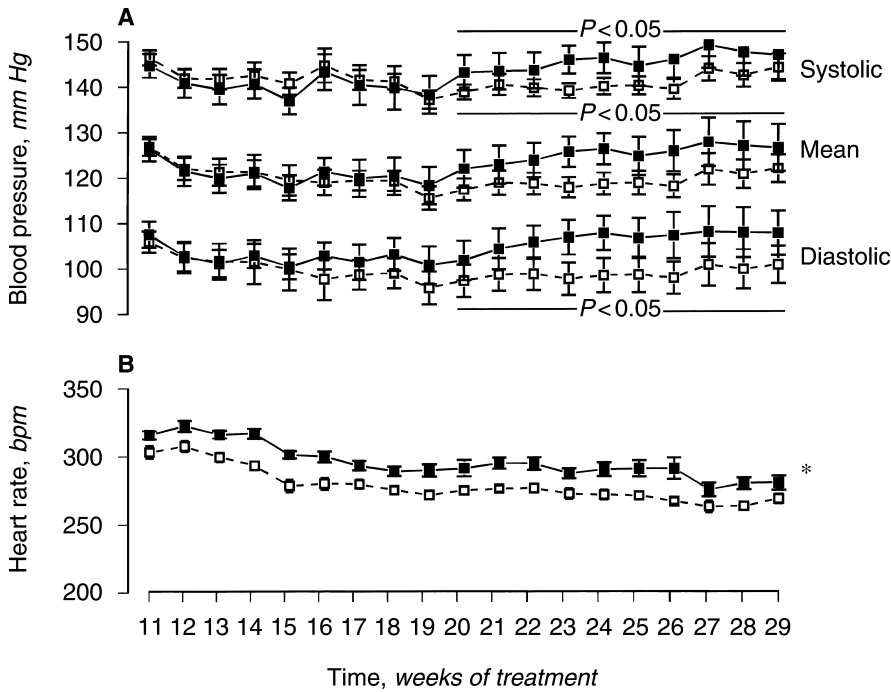Analysis of renal histology revealed a significantly

Fig. 1. Blood pressure (*A*) and heart rate (*B*) in controls (□; *N* = 6) and obese, diabetic ZSF1 rats (■; *N* = 6) consuming caffeine for 30 weeks (*P < 0.01 by 2F ANOVA).
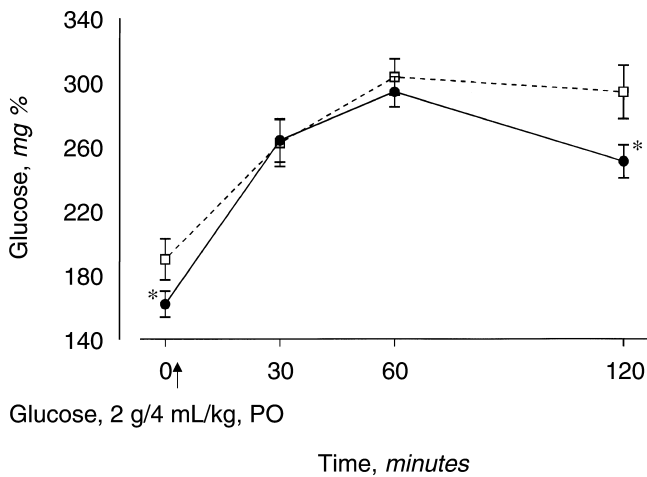

Fig. 2. Oral glucose tolerance test in controls (□; *N* = 11) and obese, diabetic ZSF1 rats after 26 weeks of caffeine consumption (●; *N* = 12). *2F ANOVA, (A) treatment, P = 0.12, (B) time, P < 0.001, A × B interaction, P < 0.002; *P < 0.05 vs. control, Fisher's LSD test.

greater incidence of glomerulosclerosis (6.5 ± 0.9 vs. 14.7 ± 1.7%, control vs. caffeine) and more severe tubulointerstitial changes in the caffeine group compared with control obese ZSF1 rats (Table 3, and Figs. 7 and 8).

DISCUSSION

The main findings of this study is that caffeine consumption for 30 weeks exacerbated renal failure and induced more severe tubulointerstitial and glomerular

damage in obese ZSF1 rats, a model that in the presence of obesity and the metabolic syndrome develops renal failure. The observed adverse renal effects of caffeine occur even in the face of improved glucose homeostasis, which would be expected to ameliorate renal function in obese, diabetic ZSF1 rats. This latter finding makes the results of this study even more significant and raises the question about possible mechanism(s) by which caffeine exacerbates renal failure in obese ZSF1 rats.

Although in the present study caffeine consumption was associated with an increase in blood pressure it is very unlikely that the exacerbation of renal failure by caffeine was due to this risk factor for renal disease [4]. Caffeine consumption increased the heart rate and produced mild, albeit significant, increases in blood pressure, suggesting that no tolerance to the hemodynamic effects of caffeine developed during chronic caffeine consumption. This is consistent with recent findings in humans. Although it has been long held that tolerance develops quickly to the hemodynamic effects of caffeine, recent studies in humans suggest that caffeine induces persistent, albeit moderate, increases in blood pressure in regular coffee drinkers [28–30], and in individuals at high risk for hypertension, this effect may become clinically significant [31, 32]. However, it should be emphasized that increases in blood pressure were modest and only occurred toward the end of the study (after 20 weeks of consumption), whereas marked effects of caffeine on proteinuria were detected after only six weeks of caffeine consumption. This suggests that the
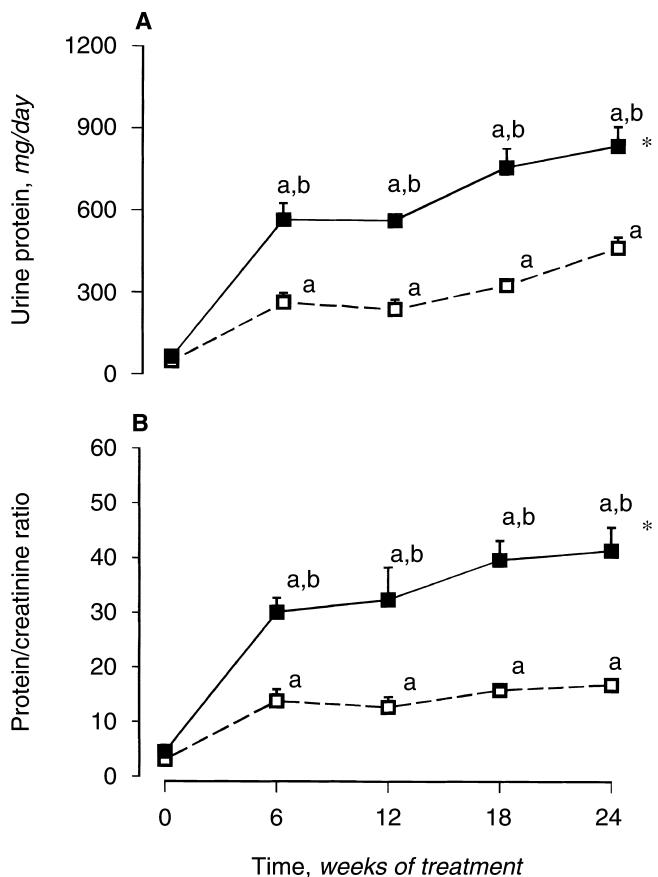
Fig. 3. Urinary protein excretion in controls (□; $N = 11$) and obese, diabetic ZSF1 rats before and after 6, 12, 18 and 24 weeks of treatment with caffeine (■; $N = 12$). *2F ANOVA, (A) treatment, $P < 0.001$, (B) time, $P < 0.001$, A × B interaction, $P < 0.001$; [a] $P < 0.05$ vs. baseline; [b] $P < 0.05$ vs. control.
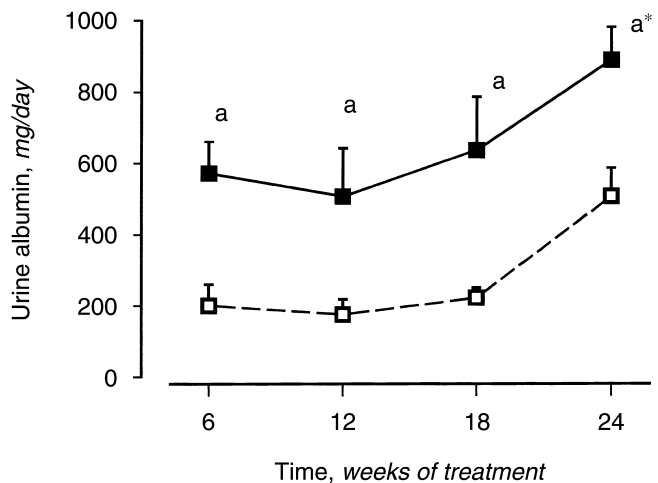

Fig. 4. Urinary albumin excretion in controls (□) and obese, diabetic ZSF1 rats (■) after 6, 12, 18 and 24 weeks of treatment with caffeine. *2F ANOVA, (A) treatment, $P < 0.001$, (B) time, $P < 0.001$, A × B interaction, $P < 0.001$; [a] $P < 0.05$ vs. control.
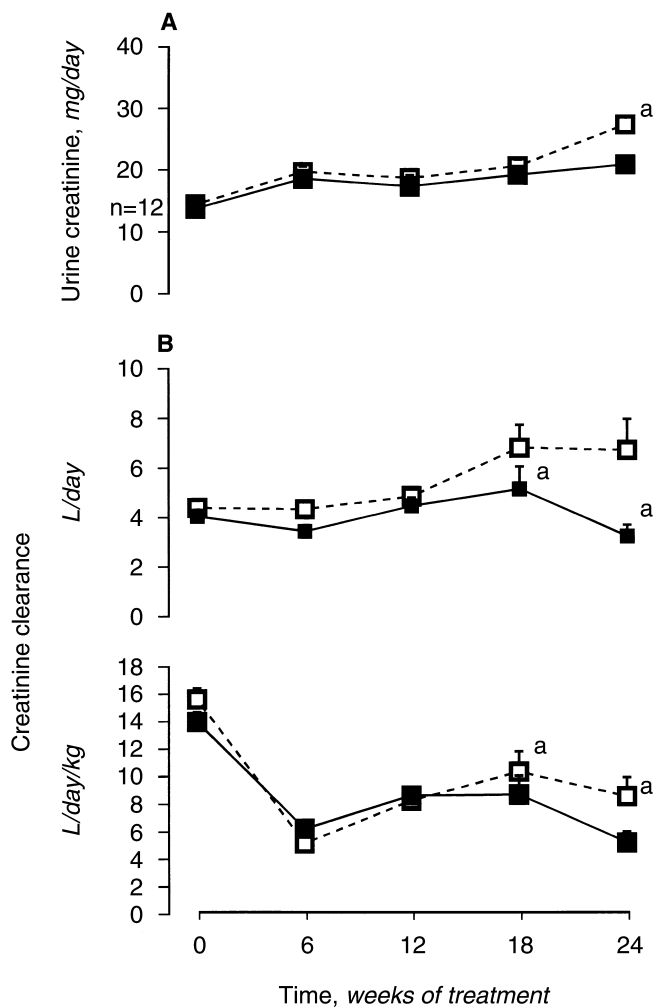

Fig. 5. Urinary creatinine excretion (A) and creatinine clearance (B) in controls (□; $N = 11$) and in obese, diabetic ZSF1 rats before and after 6, 12, 18 and 24 weeks of treatment with caffeine (■; $N = 12$). 2F ANOVA, (A) treatment, $P < 0.001$, (B) time, $P < 0.001$, A × B interaction; $P < 0.03$; [a] $P < 0.05$ vs. control.

elevated blood pressure did not cause the initial renal injury. In this regard, caffeine has been shown to augment blood pressure and exacerbate renal damage in experimental renovascular hypertension and nephropathy (2-kidney, 1 clip rats) [23, 33–36]. However, in our previous studies conducted in normotensive rats with puromycin aminonucleoside-induced nephropathy [15], spontaneously hypertensive heart failure rats [16], adult obese ZSF1 rats [17], and rats with accelerated hypertension and nephropathy induced by chronic NOS inhibition, caffeine did not alter blood pressure, yet it adversely affected renal function (abstract; Tofovic et al, *J Am Soc Nephrol* 11:631, 2000).

Caffeine significantly increased plasma cholesterol levels in obese ZSF1 rats. The available limited data regarding caffeine's effects on lipids in rats are contradictory, with studies reporting modest cholesterol increases within

Table 2. Renal hemodynamic and excretory function in obese (fa/fa^{cp}) ZSF1 rats after 30 weeks of caffeine (0.1% in drinking water) consumption

Parameters	Control	Caffeine
Body weight *g*	740 ± 11	650 ± 20[a]
Left kidney *g*	2.10 ± 0.13	2.25 ± 0.07
Mean blood pressure		
mm Hg	153.0 ± 6.1	154.1 ± 10.1
Renal blood flow		
mL/min/g kidney	3.06 ± 0.17	1.84 ± 0.21[a]
Hematocrit *%*	43 ± 1	40 ± 1
Renal plasma flow		
mL/min/g kidney	1.75 ± 0.1	1.12 ± 0.14[a]
Renal vascular resistance		
mm Hg/mL/min/g kidney	50.8 ± 2.5	95.1 ± 10.9[a]
Urine volume		
mL/min/g kidney	33.5 ± 3.7	20.4 ± 3.9[a]
Glomerular filtration rate		
mL/min/g kidney	0.97 ± 0.13	0.37 ± 0.06[a]
Plasma creatinine		
mg/dL	0.56 ± 0.11	1.33 ± 0.33[a]
Creatinine clearance		
mL/min/g kidney	0.78 ± 0.11	0.23 ± 0.08[a]
Sodium excretion		
μEq/min/g kidney	0.66 ± 0.12	0.43 ± 0.15
Potassium excretion		
μEq/min/g kidney	1.11 ± 0.10	0.79 ± 0.16
Creatinine excretion		
μg/min/g kidney	3.55 ± 0.36	1.78 ± 0.30[a]
Urinary protein excretion		
μg/min/g kidney	33.5 ± 3.6	71.8 ± 18.9[a]

Data are mean ± SE.

[a] $P < 0.05$, Caffeine vs. Control
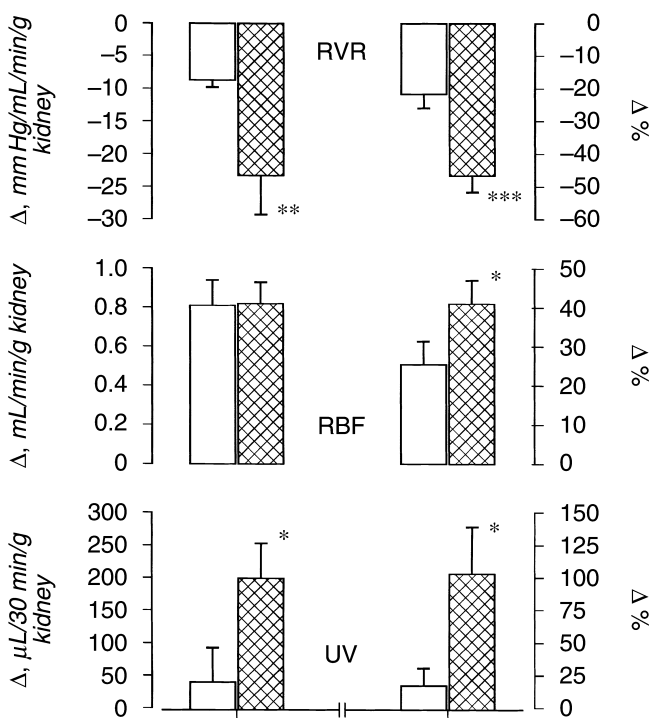
Table 3. Renal histopathology in obese (fa/fa^{cp}) ZSF1 rats treated with caffeine for 30 weeks

	Control $N = 12$	Caffeine $N = 12$	*P*
Total kidney weight *g*	4.08 ± 0.15	4.44 ± 0.21	NS
Kidney/body weight ratio *mg/g*	5.45 ± 0.22	6.98 ± 0.51	<0.05
FSGS + FGGS *%*	6.5 ± 0.9	14.7 ± 1.7	<0.002
Tubular atrophy *0–3*$^+$	0.3 ± 0.1	0.8 ± 0.2	<0.02
Tubular dilation *0–4*$^+$	1.8 ± 0.1	2.5 ± 0.2	<0.015
Interstitial inflammation *0–3*$^+$	1.2 ± 0.2	2.0 ± 0.1	<0.015
Interstitial fibrosis *0–3*$^+$	0.1 ± 0.1	0.3 ± 0.1	NS
Medial hypertrophy *0–3*$^+$	1.6 ± 0.1	1.7 ± 0.2	NS
Arteriolar sclerosis *0–3*$^+$	1.1 ± 0.2	0.9 ± 0.1	NS

the physiological range [37], transient but not sustained increases in plasma cholesterol in rats on a high cholesterol diet [38], or no effects in rats consuming boiled coffee [39]. The striking effects of caffeine on proteinuria detected after six weeks of treatment were initially accompanied by very modest increases in plasma cholesterol levels (week 8, 207 ± 7 and 259 ± 13 mg/dL, control and caffeine groups, respectively; Table 1). It is very unlikely that this modest increase in plasma cholesterol (that is, a risk factor for renal disease [40]) caused initial renal damage and induced proteinuria. More likely, similar to elevated lipids in nephrotic syndrome [41], increased cholesterol levels were due to augmented proteinuria.

Chronic caffeine consumption reduced body weight, food consumption, glycosuria, fasting glucose and insulin levels, and improved the oral glucose tolerance in obese ZSF1 rats. This is not surprising in view of the significant and tissue specific effects of adenosine on insulin sensitivity. Adenosine via activation of A$_1$ receptors decreases the sensitivity to insulin in skeletal muscle, a tissue that is considered the most important site for glucose disposal in response to insulin. Furthermore, adenosine A$_1$ receptor antagonists have been shown to increase sensitivity to insulin in skeletal muscle from lean [42] and obese rats, and to improve glucose tolerance in obese Zucker rats in vivo [43, 44]. Importantly, caffeine significantly reduced body weight. This is in congruence with our previous study conducted in adult ZSF1 rats where 8 weeks of caffeine consumption reduced food intake and body weight [17]. It appears that appetite suppression and weight reduction in response to caffeine occurs in obese but not lean animals. In this regard caffeine combined with ephedrine was shown to reduce food intake and to reverse or prevent obesity in genetically obese fa/fa Zucker rats [45, 46], and to reduce food intake and body weight in obese but not in lean primates [47]. Similarly, in our previous studies conducted in lean rats with nephropathy associated with genetic hypertension and insulin resistance or in lean rats with puromycin-aminonucleoside induced nephropathy, no effects of 24 weeks of caffeine consumption were detected on food intake or
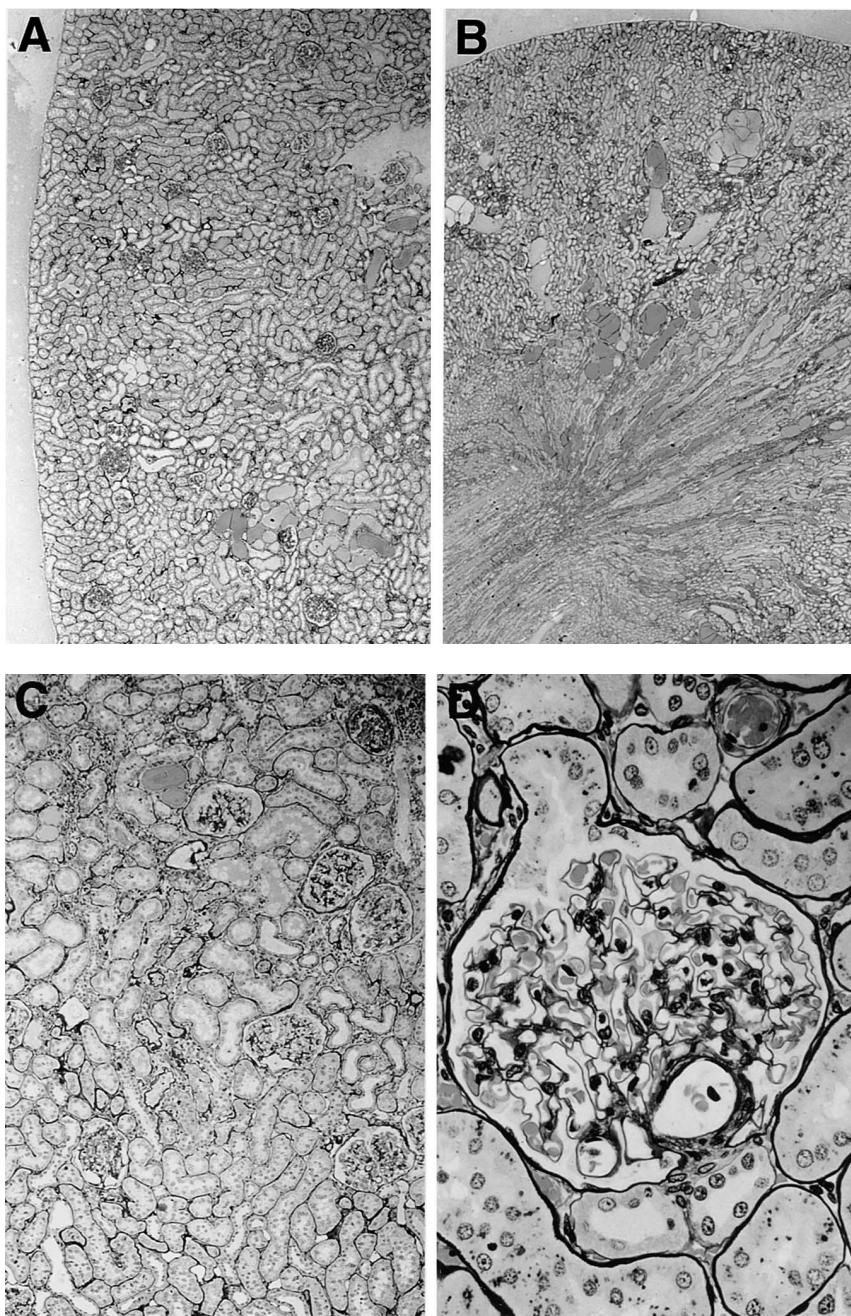

Fig. 6. Changes in renal hemodynamic and excretory function parameters after administration of captopril. Symbols are: (□) control; (⊠) caffeine. Baseline period values are presented in Table 2; * $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$.

Fig. 7. Representative light micrographs showing histological alterations in a kidney from control obese, diabetic ZSF1 rat at 38 weeks of age. (*A*) ×22; (*B*) ×44; (*C*) ×110; (*D*) ×440; all methenamine silver-trichrome stained.

body weight [15, 16]. The reduced food consumption and body weight and improved glucose homeostasis by caffeine would be expected to ameliorate renal function in obese diabetic ZSF1 rats. Nevertheless, caffeine very early (that is, after 6 weeks of caffeine consumption) doubled urinary protein excretion.

The early, striking increase in proteinuria and late changes in glomerular filtration in the caffeine group were accompanied by only a modest increase in the incidence of glomerulosclerosis. Furthermore, obese ZSF1 rats expressed only mild hyperglycemia, and caffeine

consumption actually improved glucose control. Altogether, these observations suggest that changes in creatinine clearance were more likely due to hemodynamic changes and the prolonged massive proteinuria, rather than to diabetic glomerulosclerosis. In this regard, more severe interstitial changes (that is, inflammation) and increased incidence of glomerulosclerosis in caffeine-treated animals are consistent with an early increase in protein excretion and late changes in creatinine clearance. It is believed that in overt proteinuria the filtered proteins that leak into the interstitium induce interstitial
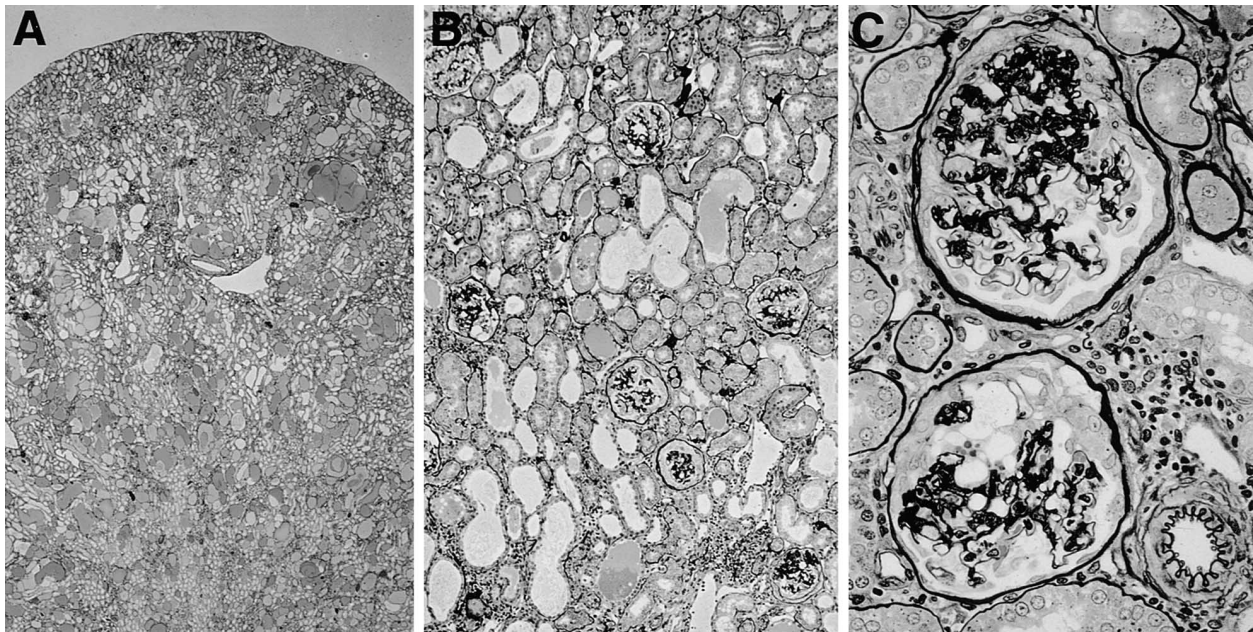
Fig. 8. Representative light micrographs showing histological alterations in a kidney from obese, diabetic ZSF1 rat after 30 weeks of caffeine consumption. (*A*) ×22; (*B*) ×110; (*C*) ×330; all methenamine silver-trichrome stained.

inflammation and a subsequent increase in extracellular matrix and scarring [48]. It is possible that the increase in glomerulosclerosis observed in the present study was due to prolonged and marked proteinuria and increased interstitial inflammation. It seems that cellular infiltration and interstitial inflammation directly contribute to the decline in renal function (that is, creatinine clearance) and determine the magnitude of proteinuria and progression of late histological changes in experimental nephropathy. In this regard, irradiation or pharmacological inhibition of interstitial inflammation improves glomerular filtration, prevents or attenuates proteinuria and diminishes the progression of histological changes in experimental nephropathy [49–51]. It should be emphasized that obese Zucker rats develop interstitial inflammation very early (5 weeks of age), which is closely associated with proteinuria but is distinctly separate from the glomerulosclerosis that occurs at an older age [52, 53]. It is possible that caffeine, via inhibition of A_{2A} adenosine receptors, accelerated the development of interstitial inflammation. For example, activation of A_{2A} receptors was shown to inhibit polymorphonuclear (PMN) cells infiltration and protect kidneys from ischemic reperfusion injury in rats (abstract; Okusa et al, *J Am Soc Nephrol* 9:583A, 1998) [54]. Also, adenosine via A_{2A} receptors inhibits leukocyte-induced vasoconstriction by preventing the adhesion of neutrophils to endothelium and superoxide generation [55]. In contrast, both caffeine and theophylline (non-selective adenosine receptor antagonist similar to caffeine) were shown to reverse adenosine receptor-mediated anti-inflammatory effects of methotrexate in vivo in rats [56]. Furthermore, at pharmacologically relevant concentrations, caffeine augments TNFα-primed activation of PMN cells [57]. It is possible that caffeine, by interacting with A_{2A} receptors, accelerates the development of interstitial inflammation and augments proteinuria and the late changes in renal function and structure. However, since the present study did not address the temporal relationship between interstitial changes and proteinuria, no definitive evidence as to whether the increased interstitial inflammation is the cause or the consequence of increased proteinuria is provided. Our study results might seem somewhat paradoxical since theophylline, a non-selective adenosine receptor antagonist similar to caffeine, has been used to improve renal function in various models of acute renal failure. However, there is compelling evidence that, in contrast to acute renal failure, in chronic renal failure adenosine may confer renoprotection, whereas adenosine antagonists may adversely affect renal function. In addition to its anti-inflammatory effects (supra vide) adenosine interacts with several other systems that play an important role in progression of renal failure, and inhibition of that interaction (that is, by caffeine) may have ill effects on renal function.

First, endogenous adenosine via activation of A_1 receptors restrains basal renin release and the renin release response to various stimuli [58], whereas caffeine augments basal renin release and renin release responses to various stimuli in experimental settings and in humans

[59–63]. In the present study, inhibition of renin-angiotensin system (RAS) with the ACE inhibitor captopril resulted in greater changes in renal hemodynamics in caffeine-treated rats, suggesting increased activity of the renal RAS in animals that were consuming caffeine. Angiotensin II is known (via preferential constriction of efferent arterioles) to increase intraglomerular capillary pressure and to stimulate glomerular growth and fibrosis [64], and chronic activation of RAS is well documented to have detrimental effects on renal function.

Second, adenosine plays an important role in modulating glomerular hemodynamics. At lower concentrations adenosine via A_1 receptors constricts the afferent arteriole, whereas at higher (micromolar) concentrations adenosine activates A_2 receptors and dilates the efferent arterioles. The activation of both types of receptors results in a marked fall in intraglomerular pressure and prevents glomerular hyperfiltration. In contrast, the inhibition of adenosine receptors may be expected to have the opposite effect. Interestingly, in micropuncture studies in rats, blockade of A_1 receptors with DPCPX, a selective A_1 receptor antagonist, reduces afferent arteriolar tone and increases intraglomerular pressure [65]. Also in rats, caffeine augments angiotensin II-induced increases in filtration fraction, suggesting that caffeine may augment angiotensin II-induced glomerular hypertension [27].

Third, several studies suggest that adenosine plays an important role in signal transmission in the tubuloglomerular feedback (TGF) mechanism. Interestingly, the homeostatic efficiency of TGF is reduced in diabetic nephropathy, and this renders the diabetic glomeruli more susceptible to fluctuations in systemic blood pressure [66, 67]. More importantly, dipyridamole (adenosine uptake inhibitor that increases interstitial adenosine levels) restores TGF activity and corrects some aspects of diabetic nephropathy (hyperfiltration, proteinuria, increased kidney weight) in streptozotocin diabetic rats [9]. In contrast, theophylline, a non-specific A_1 and A_2 receptor antagonist very similar to caffeine, and DPCPX, a selective A_1 receptor antagonist, were found to decrease the activity of TGF [68, 69]. The above discussion suggests that caffeine, by blocking the renovascular effects of adenosine and simultaneously increasing intrarenal angiotensin II levels, may permit a greater portion of the systemic blood pressure to be transmitted to glomeruli, thus causing renal injury.

Finally, in nephropathic kidneys, under conditions that are known to increase adenosine release (that is, ischemia, tissue injury, and high tubular metabolic demand due to proteinuria or glycosuria) it may be expected that an increased adenosine concentration would be detected. Interestingly, the kidneys from diabetic rats have a greater capacity to produce and maintain higher extracellular concentrations of adenosine [70]. More importantly, nephropathic kidneys form diabetic rats are more

sensitive to the vascular effects of adenosine [71]. Therefore, the inhibition of adenosine receptors by caffeine may be expected to induce more severe adverse effects in diabetic kidneys.

The above discussion examined several non-mutually exclusive mechanisms that may mediate the adverse renal effects of caffeine. Although these effects may not be significant in healthy kidneys, in pre-existing nephropathy in the presence of other risk factors caffeine may accelerate renal deterioration. In this regard, our previous studies demonstrated that caffeine consumption has adverse renal effects in several models of experimental nephropathy in rats [15–17], but has no adverse effect on renal function and structure in normotensive lean rats with intact kidneys [15] and in adult spontaneously hypertensive rats that (due to protective effects of elevated preglomerular vascular resistance) have normal renal function and develop nephropathy very late [23].

In summary, this study provides the first evidence, to our knowledge, that in the face of improved insulin sensitivity, caffeine exacerbates renal failure in the experimental setting of obesity and the metabolic syndrome. Our results imply that the health consequences of chronic caffeine consumption may be importantly determined by underlying pathophysiology, and that negative studies in normal humans do not exclude possible adverse effects in complex disease states. These data provide a strong rationale for examining the metabolic and renal effects of caffeine consumption in individuals with chronic renal failure and the metabolic syndrome.

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